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VASCULOGENESIS

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CONTENTS

ABSTRACT

Induction by fibroblast growth factors of mesoderm during gastrulation leads to blood-forming tissue, including angioblasts and hemopoietic cells, that together constitute the blood islands of the yolk sac. The differentiation of angioblasts from mesoderm and the formation of primitive blood vessels from angioblasts at or near the site of their origin are the two distinct steps during the onset of vascularization that are defined as vasculogenesis. Vascular endothelial growth factor and its high-affinity receptor tyrosine kinase flk-1 represent a paracrine signaling system crucial for the differentiation of endothelial cells and the development of the vascular system. Specific cell adhesion molecules such as VE-cadherin and PECAM-1 (CD-31), and transcription factors such as ets-1, as well as mechanical forces and vascular regression and remodeling are involved in the subsequent events of endothelial cell differentiation, apoptosis, and angiogenesis.

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Introduction

The cardiovascular system is the first functional organ system that develops in the vertebrate embryo. Embryonic growth and differentiation essentially depend on transport of nutrients and waste through the early vasculature, and certain events in morphogenesis are thought to be influenced by the hemodynamic forces of the beating heart. In the adult, the vasculature not only serves as a "nutrient and waste pipeline" but is also a major communication system between distant organs and tissues. In most tissues, the vasculature itself is highly specialized to meet the particular needs of the tissue in terms of quality and quantity of incoming and outgoing molecules and messages. This review focuses on the molecular mechanisms by which new blood vessels form in the early embryo.

The first step of blood vessel formation is the differentiation of vascular endothelial cells, which later cover the entire inner surface of all blood vessels. As soon as the early mesoderm has formed via the process of gastrulation, a subset of the primitive mesodermal cells is committed to differentiate into endothelial cells that in turn give rise to the vascular primordia of the embryo. These cells are called angioblasts. The differentiation of angioblasts from mesoderm and the formation of primitive blood vessels from angioblasts at or near the site of their origin are the two distinct steps during the onset of vascularization that are defined as vasculogenesis.

Mesoderm Formation

Of the three germ layers, mesoderm and endoderm are derived from the embryonic epiblast by the process of gastrulation. Recently, significant progress has been made in unraveling the molecular mechanisms involved in the differentiation of mesodermal cells from their epiblastic precursors. Amphibian embryos are most amenable to these studies because in a simple assay system (the animal cap assay), mesoderm is induced from the animal pole cells by the vegetal cells. With use of this assay, polypeptide growth factors have been identified that can replace the activity of the vegetal cells. Members of the fibroblast growth factor (FGF) family are potent inducers of ventral mesoderm, which in *Xenopus* embryos includes muscle and blood cells (Slack et al 1987, Godsave et al 1988, Knöchel et al 1989, Isaacs et al 1992, Tannahill et al 1992). Members of the large family of transforming growth factor-beta (TGF-β)-like factors were found to induce the mesoderm, including notochord and somitic tissue (Green et al 1992). More recently, FGFs have been shown to be necessary for the differentiation of both types of mesoderm, which suggests a synergistic action of factors during mesoderm formation (Cornell & Kimelman 1994, LaBonne & Whitman 1994). This is consistent with model systems in which gradients and local concentrations of morphogens determine the

extent and quality of the induced mesoderm. The FGF/FGF-receptor system may be the crucial signal transduction pathway for mesoderm induction in vivo because data from dominant-negative mutants and knock-out mice have provided direct evidence for the requirement of FGF-receptor tyrosine kinases (Amaya et al 1991) and of FGF-4 (Feldmann et al 1995). The member of the TGF-β family that induces axial mesodermal structures has not been identified, although the product of the *VG1* gene seems to be a good candidate (for review see Kessler & Melton 1994).

Although amphibian embryos provide an excellent model to study the molecular mechanisms of mesoderm induction, they have two disadvantages. First, the layer of cells that in an intact embryo normally would give rise to mesoderm is deleted in order to perform the cap assay. Second, and for our interest most important, detailed studies of the early development of the vascular system have not been performed, and there are no markers available for endothelial cells in amphibian embryos. Conversely, the development and morphogenesis of the vascular system has been extensively studied in avian embryos because of the ease of observation and manipulation of living embryos at the appropriate stages. In addition, the experimental advantages of chick-quail chimeras and the availability of markers for endothelial cells (LeDouarin 1973, Labastie et al 1986, Pardanaud et al 1987) make the avian embryo an excellent model to study early vascular development. In the following, we focus on the vascular mesoderm in avian species and compare it, when appropriate, to murine vascular development.

The unincubated avian egg already contains a primitive embryo that is spread disc-like on the yolk and consists of several thousand cells. This blastodisc is subdivided in two concentric zones: the inner area pellucida with the subgerminal cavity and the outer area opaca, also called the germ wall. The latter gives rise to the yolk sac ectoderm and endoderm while rapidly expanding around the yolk. The area pellucida represents the embryoblast, which consists of the pluripotent epiblast and, at the time of laying, an incomplete layer of primary hypoblast. The blastodisc is polarized in an cranio-caudal axis, the caudal pole of which is defined by a sickle-shaped condensation of cells at the margin of the central embryoblast and the peripheral area opaca (Eyal-Giladi & Kochav 1976, Kochav et al 1980). At this so-called Koller's sickle, a small number of cells positioned between the epiblast and the primary hypoblast express the homeobox-gene *goosecoid* (Izpisua-Belmonte et al 1993). These cells are capable of inducing a secondary axis when ectopically transplanted into a host embryo and are therefore believed to trigger the process of gastrulation. In birds, mammals, and some reptiles, this comprises invagination of the epiblast through the primitive streak along the cranio-caudal axis (Bellairs 1986) (Figure 1). The cells that remain in the epiblast will form the nervous system, the neural crest (mesectoderm), and the ectodermal epithelia. The cells

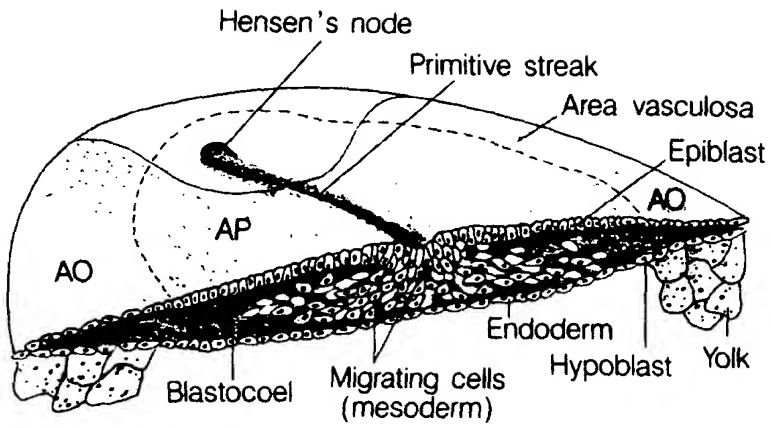
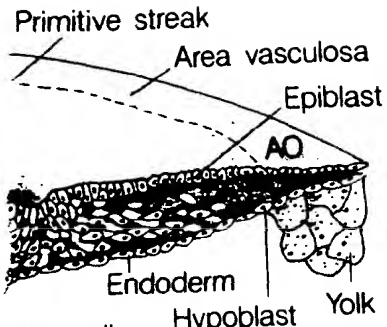


Figure 1 Schematic view of mesoderm differentiation and migration in a gastrulating chick embryo (about 17 h of incubation). The stippled area indicates the area vasculosa. AP: area pellucida; AO: area opaca

that leave the epiblast through the primitive streak give rise to both the definitive hypoblast (endoderm), which later will contribute to the epithelium of the intestine, and the mesoderm, which initially consists of fibroblast-like migrating cells (Ebendal 1976, England & Wakely 1977). The mesodermal cells give rise to the elements of the cardiovascular system and to most of the mesenchyme and the axial structures. The peripheral cells of the early mesoderm invasively migrate outward between ectoderm and endoderm of the yolk sac and form the extraembryonic yolk sac mesoderm (Figure 1) (Mayer & Packard 1978, Flamme 1987).

Blood islands are the earliest discernible vascular structures that give rise to a primitive vascular network in the yolk sac (Gonzalez-Crussi 1971). The vascularized part of the yolk sac is called area vasculosa and is congruent with the part containing the mesodermal layer. Only two regions in the yolk sac are transiently devoid of blood vessels: (*a*) the area beyond to the head (proamnion), where the advancing edges of the lateral yolk sac mesoderm do not meet before the second day of incubation (Ravn 1886); and (*b*) the region of primitive streak regression at the embryonic tail (sinus rhomboidalis) (Pardanaud et al 1987) (Figure 2).

At the time blood islands form, the mesodermal layer becomes gradually split by the advancing extraembryonic coelom, which is contiguous with the intraembryonic coelom (Kessel & Fabian 1985). The mesodermal cells residing on the endoderm are known as splanchnopleuric mesoderm, whereas those



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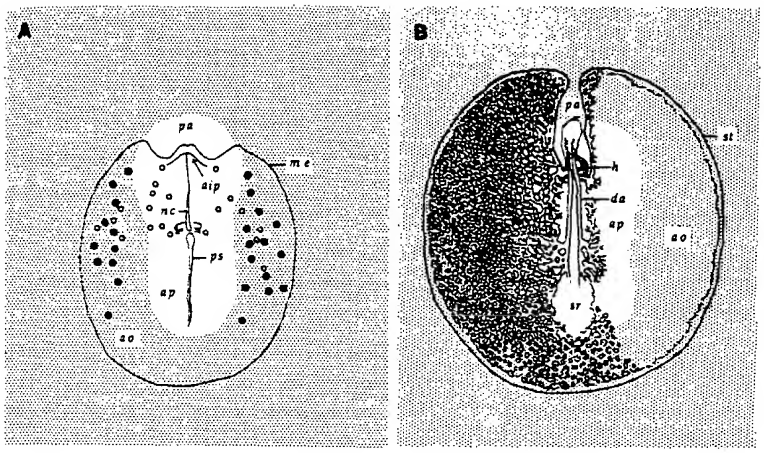


Figure 2 Features of vasculogenesis in the avian embryo. (*A*) Schematic drawing of the distribution of blood islands (*dots*) consisting of hemopoetic cells and angioblasts, and solitary angioblasts (*circles*) at the 1-somite stage as visible by immunohistochemistry and electron microscopy The mesoderm originating from the primitive streak (ps) invades the area opaca (ao), which has already spread over the yolk; me: free edge of the expanding mesoderm. A region in front of the head fold, which forms the anterior intestinal portal (aip), is devoid of mesoderm and is called proamnion (pa) At this stage blood islands begin to interconnect and form the primary vascular plexus within the area opaca (ao). In the area pellucida (ap), only angioblasts without hemopoietic cells are present, nc: notocord.

(*B*) The vascular plexus of a chicken embryo at early day 2 of incubation (only the left side of the area vasculosa is drawn in detail) as formed by vasculogenesis. The edge of the expanding mesoderm extends just beyond the outermost vessel, the marginal sinus (st). Extraembryonic mesoderm and the vascularized part of the area opaca (ao) are congruent and called area vasculosa. At this stage the heart (h) is already beating and pumps blood into the circulation via the dorsal aortae (da) and the yolk sac arteries. The proamnion (pa) and the zone of primitive streak regression (sinus rhomboidales: sr) are still avascular. At this stage the head is already colonized by angioblasts

beneath the ectoderm are called somatopleuric mesoderm. Interestingly, blood island formation and primary vascularization are only observed in the splanchnopleuric mesoderm. Therefore, vasculogenesis has been thought to be regulated negatively by the ectoderm and positively by the endoderm, respectively (Augustine 1981, Kessel & Fabian 1986, 1987). There is ample evidence that gastrulation itself is not necessary for vascular mesoderm differentiation. Under experimental conditions designed to inhibit gastrulation via the primitive streak, a process called polyingression is sufficient to give rise to mesoderm, which subsequently differentiates to blood islands (Azar & Eyal-Giladi 1979, Zagris 1980). However, angioblast differentiation is not just a default pathway of mesodermal cells because removal of the yolk sac endoderm results in failure of angioblast differentiation (see below) (Wilt 1965). It was therefore

hypothesized that yolk sac endoderm-derived factors are necessary for blood island maturation (Miura & Wilt 1969) and subsequent vascular growth in the area opaca (Flamme 1989).

Blood Islands and the Hemangioblast

Blood islands are aggregates of cells that emerge from the splanchnopleuric mesoderm of the area opaca (Figure 3). The peripheral cells are the precursors of endothelial cells called angioblasts (His 1900), whereas the cells in the center of a blood island are hemopoietic precursor cells. Angioblasts are defined as a cell type that has certain markers characteristic of an endothelial cell (but not yet all markers) (Figure 3) and has not yet formed a lumen. The close association of hemopoietic and endothelial precursor cells has led to the assumption that endothelial cells and hemopoietic cells may have a common precursor called the hemangioblast (His 1900). This is supported by the observation that many molecules present in endothelium are also expressed by hemopoietic cells such as QH1 and MB1 in the quail (Labastie et al 1986, Pardanaud et al 1987) and cd34 (Fina et al 1990) and PECAM-1 (CD31) (Newman et al 1990) in the mouse.

Formation of the hemangioblastic cell lineages has been observed as a spontaneous event in the avian epiblast cultured in vitro (Murray 1932, Azar & Eyal-Giladi 1979, Zagris 1980) or when fragments of epiblast were heterotopically transplanted (Christ et al 1991). In these experiments, blood and endothelium-forming mesoderm differentiated independently of gastrulation, whereas axial mesoderm was found to be inducible by axial mesoderm-inducing factors. Therefore, it was concluded that committed hemangioblastic precursors are already present in the epiblast prior to gastrulation (Mitrani et al 1990). However, we have recently demonstrated that after dissociation the cells of the pregastrulation avian epiblast can be induced by FGFs to form in vivo-like blood islands in vitro, whereas no differentiation is seen in the absence of FGF even in the presence of serum (Flamme & Risau 1992). This is in contrast to mouse models of vasculogenesis using embryonic stem cell-derived cystic embryoid bodies in which blood islands differentiated spontaneously (Risau et al 1988, Wang et al 1992). The contradictory results may be due to the cell line characteristics of the mouse embryonic stem cells or to the presence of sufficient endogenous FGF-like factors. Avian blastodisc-derived cells cultured in suspension give rise to structures very similar to the mouse cystic embryoid bodies. However, in contrast to the mouse model, the avian embryoid bodies are devoid of blood vessels in the absence of exogeneous FGF. Upon induction, vascular networks form that are indistinguishable from the primitive in vivo vascular plexus, which is established during vasculogenesis in the yolk sac (Krah et al 1994).

Although epiblast cells in the in vitro avian model express FGF receptors

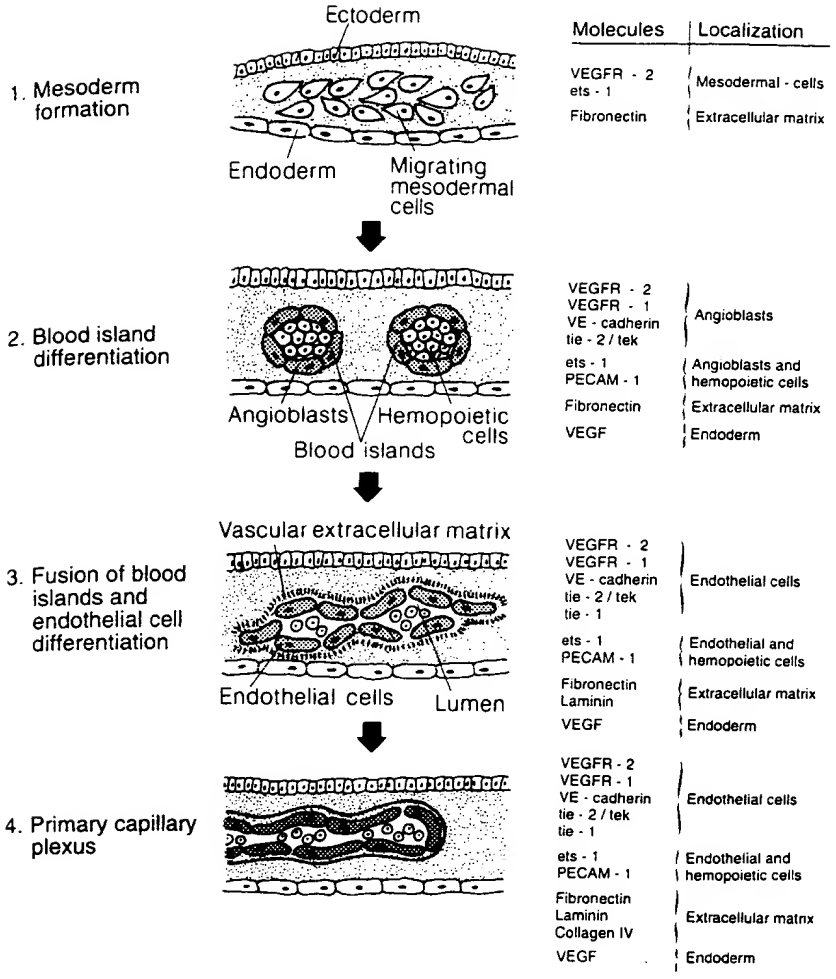


Figure 3 **Schematic representation of the processes occurring during vasculogenesis.**

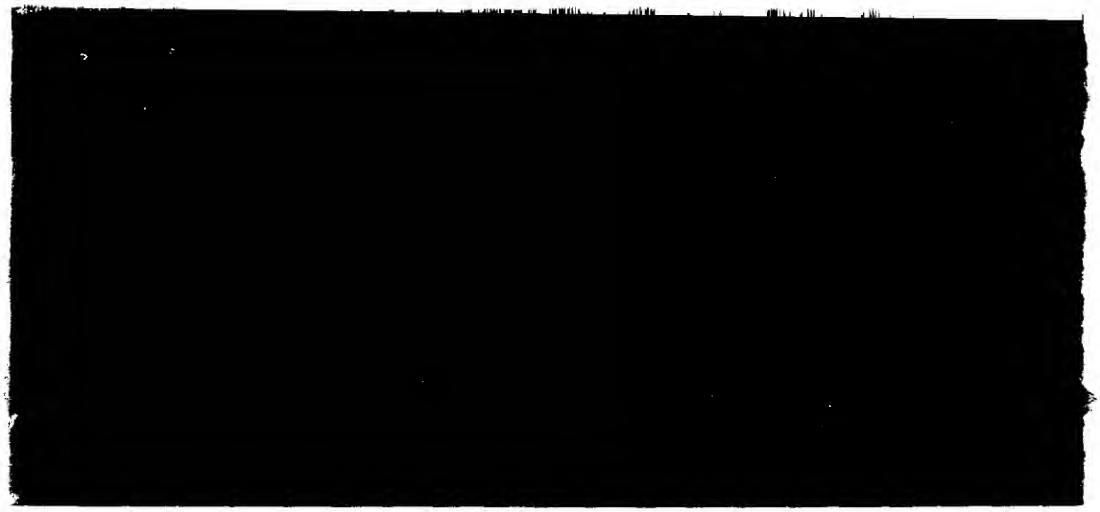
for many days (I Flamme, unpublished results), they become refractile to forming endothelial cells and blood islands after the first 24 h of in vitro culture (Flamme & Risau 1992). The reason for this change of responsiveness is unknown but is reminiscent of other cell lineage commitments during development, e.g. the time-dependent responsiveness of O2A progenitor cells to platelet-derived growth factor (PDGF) (Hart et al 1989).

Intraembryonic Angioblasts

Shortly after their extraembryonic counterparts appear, the first intraembryonic angioblasts are seen at the 1-somite stage at the lateral edges of the anterior intestinal portal, and ventral to the somites, in close contact with the endoderm (Coffin & Poole 1988). From here, angioblastic strands are formed along the lateral edges of the somites, which represent the primordia of the dorsal aortae. Already at the 2-somite stage the interconnection between extraembryonic and intraembryonic vascular primordia is established. Cranial to the anterior intestinal portal, the endocardial primordia and the ventral aorta are formed at the midline. The initially avascular head mesenchyme, which is essentially derived from the neural crest (mesectoderm), is subsequently colonized by angioblasts derived from the cephalic mesoderm. The major events of embryonic vascular pattern formation, described extensively in the classical literature, and the special problem of head-mesenchyme vascularization have been reviewed in more detail by Noden (1991).

There is a major difference between extraembryonic (area opaca) and intraembryonic (area pellucida) vascular development. In the area opaca, endothelial cell differentiation occurs concomitantly and in close association with hemopoietic precursor cells in the blood island (His 1900, Sabin 1920, Gonzalez-Crussi 1971, Pardanaud et al 1989). Within the embryo, endothelial cells differentiate from the mesoderm as solitary angioblasts without the concomitant differentiation of hemopoietic cells, except for a small region of the aorta (paraaortic clusters) (Cormier et al 1986, Olah et al 1988). Angioblasts then either migrate and fuse with other angioblasts and capillaries or form a vessel in situ. Hemopoietic cells derived from either the yolk sac or the paraaortic clusters then populate these blood vessels. The definitive hematopoietic organs are colonized by hemopoietic stem cells derived from the paraaortic region but not from the yolk sac. This has been demonstrated by the technique of yolk sac chimeras between quail and chick (Dieterlen-Lièvre et al 1975, 1988) and in the mouse by the method of "long-term reconstitution" (Müller et al 1994). The mechanisms that determine the differentiation of hemopoietic cells in blood islands and paraaortic clusters, but prevent it in the other parts of the embryo, are unknown.

There have been controversies about the origin of endothelial cells in the embryo proper. His (1900) believed that all embryonic endothelial cells immigrated from the extraembryonic yolk sac. Reagan and others (Reagan 1915, McClure 1921, Wilms et al 1991) showed by microdissection and transplantation experiments that embryonic mesoderm would give rise to endothelial cells. The conclusion from these and other transplantation experiments in the chick-quail system (Coffin & Poole 1988) is that all embryonic splanchnopleuric mesoderm, as well as somitic mesoderm, can give rise to angioblasts



and vasculogenesis. In some studies, angioblasts have also been found in the somatopleuric mesoderm (Feinberg & Noden 1991, Pardanaud & Dieterlen-Lièvre 1993). These cells might have been committed to differentiate to angioblasts before the mesoderm split; however, they constitute a minute fraction (Pardanaud & Dieterlen-Lièvre 1993) that may also be inhibited to form vessels in the vicinity of the ectoderm. Thus in situ differentiation of endothelial cells occurs primarily from mesodermal cells in contact with endoderm. In addition to the results from transplantation studies and experimental removal of area opaca endoderm (Wilt 1965), this concept is supported by several other observations: (*a*) Angioblasts also differentiate from the mesodermal precursors within endodermal organ rudiments such as lung and pancreas (Pardanaud et al 1989). (*b*) The amnion consisting of ectoderm and mesoderm is avascular, whereas an abundant vascular network develops from yolk sac and allantois, which consist of endoderm and mesoderm (Pardanaud & Dieterlen-Lièvre 1993a). It follows that organs and tissues devoid of endoderm must be vascularized by blood vessels sprouting from preexisting vessels, i.e. angiogenesis.

Angioblasts in the area pellucida seem to be very motile cells. They migrate as single cells or cell clusters and are incorporated into vessels that have formed in situ elsewhere or they form vessels themselves in locations other than their site of origin (Sabin 1920, Coffin & Poole 1988, Poole & Coffin 1989). Noden, using ectopic transplantations of pieces of early mesoderm in the chick-quail system, has shown that this migratory process is more evident in the head region than in the trunk, which suggests that the head region, which is avascular early on, may produce motility or chemotactic factors for angioblasts (Noden 1989, 1991).

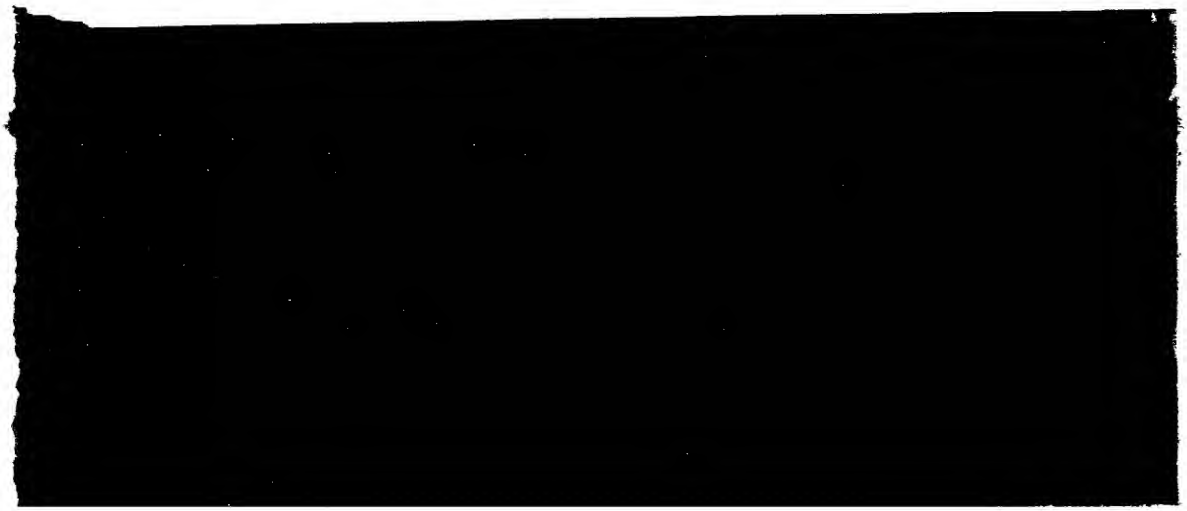
Blood island induction and subsequent blood vessel formation would therefore include the following steps (Figure 3):

1. contact of either migrating, gastrulating, or polyingressing cells bearing FGF-receptors with FGF-producing cells; the latter presumably primary hypoblast cells;
2. activation and signal transduction of the FGF-receptor leading to the activation of genes (see below);
3. aggregation of mesodermal cells adjacent to the endoderm (splanchnopleuric or visceral endoderm);
4. differentiation of angioblasts (area pellucida) or angioblasts and hemopoietic cells (area opaca and paraaortic clusters) either as common precursor (hemangioblast) or as two lineages;
5. differentiation of endothelial cells, lumen formation, and basal lamina production;
6. further growth and migration of endothelial cells and connection (bridging) of blood islands leading to a primary capillary plexus.

Role of Endothelial Growth Factors

In contrast to the crucial function of FGF-receptors in mesoderm induction, these receptors do not play a major role in the subsequent morphogenesis of the vascular system. Capillary endothelial cells in embryonic tissues and organs do not express detectable levels of mRNA-encoding FGF-receptors (Heuer et al 1990, Wanaka et al 1991, Peters et al 1992). Endothelial cells of larger vessels do express FGF-receptors and respond to FGF in vivo (Lindner et al 1990, Peters et al 1992, Liaw & Schwartz 1993). This is probably important for regenerative processes but not for vasculogenesis and angiogenesis. Angiogenesis, the sprouting of capillaries from preexisting vessels, is probably the predominant mechanism of blood vessel formation in later stages of embryonic development and in the adult (Folkman & Shing 1992). FGFs were previously thought to be important angiogenic factors, but direct in vivo evidence is still lacking. In most of the in vivo angiogenesis model systems, e.g. rabbit cornea and chick chorioallantoic membrane, FGFs seem to act indirectly (Knighton et al 1990). In contrast to vascular endothelial growth factor (VEGF), which is sufficient for the formation of new blood vessels if overexpressed in vivo (Flamme et al 1995b), FGFs do not induce new blood vessel formation (Riley et al 1993). The role of other factors such as PDGF, platelet-derived endothelial cell growth factor (PD-ECGF), tumor necrosis factor (TNF), TGF-β, TGF-α, and epidermal growth factor (EGF) is not clear (Risau 1990, Folkman & Shing 1992).

The notion that VEGF is an important regulator of embryonic and adult blood vessel development is supported by the observation that the first molecule known to be expressed in a population of mesodermal cells giving rise to angioblasts is the vascular endothelial growth factor receptor-2 (VEGFR-2; also known as flk-1 in the mouse and KDR in the human). This receptor has been detected in 7 day mouse embryos and 20 h quail embryos. Later during embryonic development, this molecule becomes restricted to endothelial cells (Eichmann et al 1993, Millauer et al 1993, Yamaguchi et al 1993, Flamme et al 1995a). In the in vitro system using avian epiblast cells, VEGFR-2 is maximally induced as early as 24 h after incubation with FGF (Flamme et al 1995a), which suggests that one of the earliest signaling events of the FGF-receptor is the activation of the VEGFR-2 gene. These cells are then likely to respond to VEGF, which is expressed in the endoderm of the 7.5 day mouse and at the definitive primitive streak stage in quail embryos (Breier et al 1995, Flamme et al 1995a). Because the endoderm is adjacent to the mesoderm, a paracrine relationship between the two germ layers may exist, and VEGF secreted by the endoderm may support the differentiation of VEGFR-2-expressing mesodermal cells to angioblasts (see Figure 3). If a threshold concentration of VEGF is necessary to sustain VEGFR-2 expression, the receptor



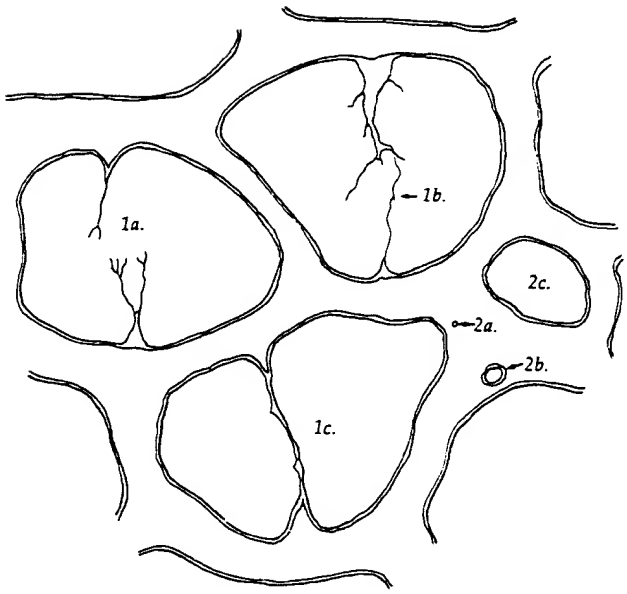


Figure 4 Scheme of the morphological steps of sprouting (angiogenic: *1a-c*) and non-sprouting (intussusceptive: *2a-c*) vascular growth as seen in the yolk sac (extraembryonic mesoderm) of the quail embryo after immunohistochemical staining of the endothelium in whole mount preparations (adapted from Flamme et al 1992, 1993). (*1a*) Sprouts bearing long filopodia at their tips extend from the endothelial lining of opposite preexisting capillaries. (*1b*) Filopodia meet each other and form a solid strand (*1c*), which splits the intervascular space. In some sprouts lumina are already visible. In the process of intussusception, a solid mesenchymal pillar grows into a capillary (*2a*), subsequently enlarges (*2b*), and forms a new intervascular space (*2c*) indistinguishable from that generated by sprouting.

may be downregulated in cells not receiving sufficient signals, and endothelial cells may not differentiate. Hemopoietic cells differentiating from the putative VEGFR-2-expressing hemangioblastic precursor cell would also be expected to downregulate this receptor. The mechanism involved in this downregulation, which probably occurs in blood islands and paraaortic clusters, is unknown.

The biological function of VEGF in vasculogenesis is not clear. It could act as a true growth factor, inducing an increase in endothelial cell number, or as a survival factor. It could also act as a lumenizing factor by virtue of its ability to increase vascular permeability, which is often associated with dilated vessels (Ferrara et al 1991, Dvorak et al 1992, Flamme et al 1995b). After formation of a primary capillary plexus, endothelial cells extend filopodia and sprout from the plexus (i.e. angiogenesis), leading to a mature vascular plexus

(Flamme 1989, Flamme et al 1993, Young et al 1995) (Figure 4). VEGF may induce this sprouting of endothelial cells from a preexisting capillary plexus.

Growth of endothelial cells can also occur within embryonic capillaries giving rise to an enlargement of lumen diameter, eventually leading to a large vessel. Alternatively, the vessel may split to form two vessels. This mode of new blood vessel formation is called intussuceptive growth and was observed in the lung, yolk sac, and chorioallantoic membrane (Burri & Tarek 1990, Flamme et al 1992, Patan et al 1993) (Figure 4).

Endothelial cell proliferation is high during embryonic and postnatal development but is very low in the adult under normal physiological conditions (Engerman et al 1967). The transient expression of VEGF and VEGF-receptors in most tissues correlates with the rapid growth of endothelium, although constitutive expression was observed in some sites (Breier et al 1992, 1995b; Millauer et al 1993). Members of a family of "orphan" receptor tyrosine kinases (i.e. the ligand is unknown) are specifically expressed in endothelial cells. These include receptors encoded by the *tie-1* (*tie*) and *tie-2* (*tek*) genes. Their expression correlates with the formation of new blood vessels, but their functions are not known. Recent experiments deleting these genes in mice demonstrate that their function is crucial for vascular development (Dumont et al 1992, 1994; Partanen et al 1992, Sato et al 1993, Schnürch & Risau 1993; T Sato, personal communication).

The Role of Oncogenes and Transcription Factors

Endothelial cells have been immortalized by different oncogenes, but they are particularly susceptible to the action of the polyoma virus middle T oncogene. In transgenic mice expressing this oncogene, endothelial cells become transformed even at the angioblast stage of vasculogenesis, and no functional vascular plexus is formed. These lethal hemangiomas or vascular malformations can also be induced later in embryonic development by recombinant retroviruses or endothelioma cells derived from the lesions. The molecular mechanism of endothelial transformation is unknown but is probably based on the constitutive activation of nonreceptor tyrosine kinases by the oncogene leading to aberrant signaling in endothelial cells. Elucidation of this mechanism may help to better understand endothelial-specific functions and signal transduction (Wagner & Risau 1994).

Another factor originally characterized as an oncogene is the ets-1 transcription factor. Although expressed in many cell types during embryonic development, a striking distribution of ets-1 mRNA was found in splanchnopleuric, but not in somatopleuric, mesoderm during vasculogenesis (Pardanaud & Dieterlen-Lièvre 1993b). A high level was detected in the peripheral angioblasts of the blood islands, as well as in intraembryonic angioblasts and endothelial cells. Later during development, the expression ceased. The ex-

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pression of ets-1 in embryonic cells including angioblasts is consistent with the notion that angioblasts in a transitional or migratory state differentially activate a set of genes. Binding motifs for ets-1 are present in the promotor regions of genes encoding metalloproteinases, which are thought to be important for extracellular matrix degradation during new vessel formation (Pepper & Montesano 1990). It is interesting to note that there is an overlapping expression of VEGFR-2 and ets-1 during vasculogenesis that is resumed and upregulated during tumor angiogenesis in the adult (Wernert et al 1992, Plate et al 1993).

Role of Cell Adhesion Molecules

Vascular endothelial cadherin (VE-cadherin; also known as cadherin-5) (Lampugnani et al 1992), the platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31) (Baldwin et al 1994), and cd34 (Fina et al 1990, Young et al 1995) are cell-cell adhesion molecules expressed very early in angioblasts. Although PECAM-1 and cd34 are also present in megakaryocytes, platelets and some other predominantly hemopoietic cell types, VE-cadherin is most specific for endothelium (G Breier et al, submitted). VE-cadherin and PECAM-1 are probably involved in homotypic endothelial cell adhesion and in the formation of interendothelial junctions, which are important for lumen formation, cell polarity, and vascular permeability. The function of the mucin-like cd34 molecule in embryonic capillaries is unknown. Adhesion molecules like ICAM-1, VCAM, and many different integrins are expressed on a wide variety of cell types, but some may be more selectively expressed in the vascular system in some conditions, e.g. after cytokine activation or during developmental processes (e.g. E-selectin; Bevilacqua et al 1989, Nguyen et al 1993) or in an organ-specific endothelium. During vasculogenesis, fibronectin and its receptor $\alpha5\beta1$ integrin are required for vascular development because their deletions result in early lethal vascular defects (George et al 1993, Yang et al 1993). This is consistent with the abundant presence of fibronectin in blood islands and the capillary plexus (Risau & Lemmon 1988, Poole & Coffin 1988). In addition, the β-1 integrin is important for vasculogenesis and lumen formation of the dorsal aorta (Drake et al 1992). Other integrins like $\alpha2\beta1$ (Languino et al 1989) seem to be expressed in endothelium, but their functions are unknown. More recently the integrin $\alpha V\beta3$ was found to be upregulated in angiogenic endothelium and seems to play a role in tumor angiogenesis (Brooks et al 1994). Laminin, vitronectin, and other extracellular matrix molecules are produced by endothelial cells later during vasculogenesis, and an intact basal lamina is a characteristic feature of a mature blood vessel (Bär & Wolff 1972, Murphy & Carlson 1978, Risau & Lemmon 1988, Herken et al 1989). There is evidence that some laminin isoforms are expressed more selectively in endothelial cells (Sorokin et al 1994). These molecules may not only have important functions in cell adhesion but also in the

storage, accumulation, and activation of proteases and protease inhibitors such as urokinase and plasminogen activator inhibitor-1, and growth factors such as VEGF and cytokines (Tanaka et al 1993).

Role of Mechanical Forces

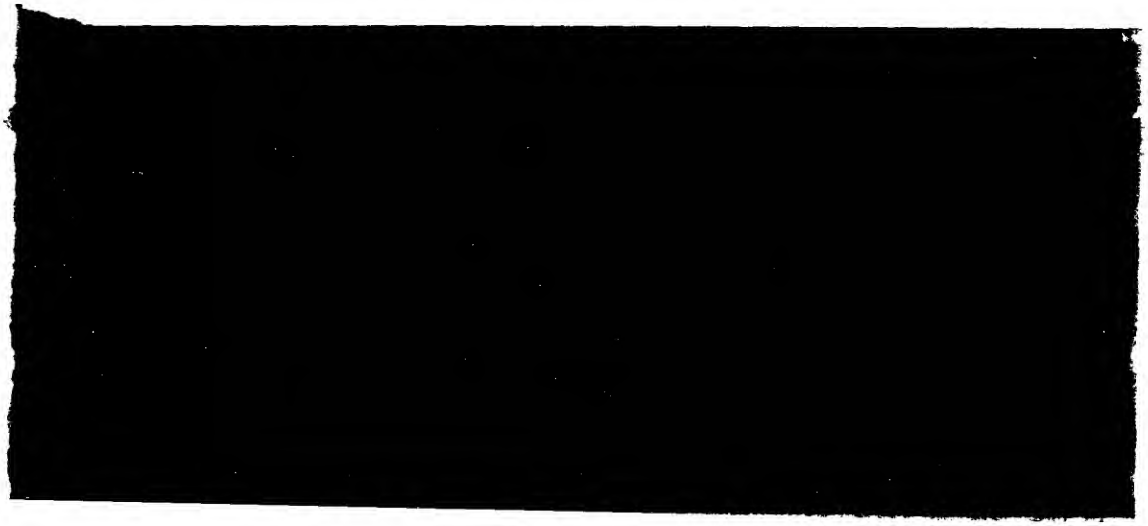
Shear stress is a major mechanical force that endothelial cells must withstand. It develops after the onset of circulation and has a major impact on the remodeling and further development of the vascular system (Chapman 1918, Thoma 1893). For example, large blood vessels in the yolk sac only develop after the vascular plexus has connected with the intraembryonic vessels via the vitelline arteries and veins. In fact, failure of this connection, as observed in retinoic acid–deficient quails, results in the complete regression of blood vessels in the yolk sac and death of the embryo (Heine et al 1985). However, not all large vessels are dependent on circulation for their development. The heart tubes, the aortic arches, the dorsal aortae, and cardinal veins are present as large vessels before the onset of circulation and shear stress.

Shear stress response elements have recently been characterized in the promotors of many genes. The PDGF gene contains such an element and is upregulated in the vascular endothelium in response to shear stress (Resnick et al 1993). PDGF and other growth factors may be involved in the regulation of the vascular wall, which has to withstand the mechanical forces exerted by the blood stream. In addition, the angiotensin system and vasoconstrictive and vasodilatory factors are involved in the physiological regulation of vascular tone. However, these factors probably play a role rather late during vascular differentiation.

Role of Vascular Regression and Apoptosis

Most likely only a minority of the blood vessels formed during embryonic development persist until the adult stage. Most capillaries in a primitive vascular plexus regress. Blood flow seems to be one determinant because unperfused capillaries seem to regress preferentially during embryonic development (Herre & Thompson 1985). However, the vascular system is laid down before the onset of circulation. This raises the possibility that endothelial cells before and after the onset of circulation respond differently to changes at their luminal surface, e.g. blood flow.

Capillaries in prechondrogenic areas also regress to allow cartilage differentiation (Feinberg et al 1986, Latker et al 1986, Hallmann et al 1987). The hyaloid vascular system regresses to allow the development of a translucent vitreous body compatible with vision (Latker & Kuwabara 1981). There is evidence for endothelial cell death (apoptosis) in these systems, but the molecular mechanisms are unknown. Another example is the regression of the primordial aortic arches, which only transiently form in situ in mammals,



although they persist in some fish species (Hahn 1909). Regression involves endothelial cell death and plays a major role in the early development of the vascular system. There is evidence that endothelial cell death is under stringent control of the local environment irrespective of the origin of endothelial cells. Genetically programmed endothelial cell death is unlikely to occur in the majority of vascular regression processes.

Concluding Remarks

According to Sabin, one of the outstanding pioneers in the analysis of early vascular development, there is a fundamental difference in the determination of a vessel, depending on whether it differentiates in situ or develops from preexisting vessels (Sabin 1917). The differentiation of angioblasts from mesoderm and the formation of primitive blood vessels from angioblasts at the site of their origin are the two distinct steps in early embryonic vascularization, defined as vasculogenesis. Thereby a vascular system is laid down before it is used for its main function, the nourishment of the fast-growing embryo. Conversely, blood vessels in the adult form in direct response to tissue demands and seem to form predominantly, if not exclusively, by sprouting from preexisting vessels. In the past decade there has been a tremendous advance in the understanding of molecular mechanisms regulating vasculogenesis and angiogenesis in the embryo. Recent results have had a great impact on insights into the mechanisms of new blood vessel formation during physiological and pathological processes.

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